α-P^{32} dCTP (3000 ci/mmol) in conjunction with the MegaPrime labeling-kit from Amersham. Specific activities of 5 X 10^9 cpm/µg were routinely obtained. Hybridization (60° C), and prehybridization (60°C), of blots were carried out using published methods (Rowe, Hum. Genet. 97 (1996), 354-352), and stringency washes were carried out as follows: 1; two washes at room temperature for 30 min with 2X SSC 0.1%SDS, two washes at 60° C for 30 min in 0.1X SSC 0.1% SDS. Filters were then exposed to film for 7 days at -80C and the films developed. Total human-RNA from adrenal glands, brain, duodenum, heart, kidney, liver, lung, skin, spleen, thymus, thyroid, tonsil, did not amplify using RT/PCR and MEPE specific primers, although evidence for low level expression using cDNA template was found for brain, kidney, liver and pancreas. For this experiment, total RNA was extracted from the following human tissues: 1; Thymus, 2; brain, 3; testis, 4; duodenum, 5; heart, 6; skin, 7; liver, 8; tonsil, 9; spleen, 10; thyroid, 11; adrenal, 12; lung, 13; kidney, 14; OHO-tumour tissue, 14; Human primary osteoblast. Total RNA from Rat primary osteoblast was also obtained. MEPE Internal primers as described above (Pho433-111F and PHO877-111R), were used to copy total RNA using reverse transcriptase-PCR and the Perkin Elmer-Roche RNA PCR kit. Briefly, 1 ug of total RNA was dissolved in 20 µl of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 5 mM $MgCl_2$, 1 mM dNTPs, 1 unit/µl ribonuclease inhibitor, 2.5 unit/µl MULV reverse transcriptase, 0.75 µM down stream primer (PHO877-111R). The mixture was then incubated at 37° C for 10 min. Upstream primer (Pho433-111F), dNTPs, $MgCl_2$, and AmpliTaq DNA polymerase, was then added to give final concentrations of 0.15 µM, 200 µM, 2 mM, and 2.5 units/100 µl respectively in a total volume of 100 µl. PCR was then carried out using a Perkin Elmer thermocycler (system 9700), set to the following program: predenaturation; 95°C 3 min; followed by thirty five cycles of denaturation; 95°C 45 sec, annealing; 65°C 30 sec, polymerization; 72°C 45 sec, and a final extension of 72°C 7 min followed by cooling to 4°C. Amplified products were resolved using agarose-gel electrophoresis, and verified by southern blotting, and sequencing. Also, a panel of normalized cDNA's derived from a range of non-OHO tumours (Breast carcinoma, lung carcinoma I, colon adenocarcinoma I, lung carcinoma II, prostatic adenocarcinoma, colon adenocarcinoma II, ovarian carcinoma, pancreatic

carcinoma; Clontech human-tumour panel #K1422-1) were all negative to MEPE PCR, except for very low level expression in one case of colon adenocarcinoma, ovarian carcinoma, and prostatic carcinoma respectively (detected after southern screening of RT/PCR amplified products with radiolabeled MEPE cDNA). In sharp contrast, RT/PCR using MEPE primers amplified poly A+ RNA, from OHO tumours, from four separate patients BD, DM, EM, and DS, indicating high levels of expression (normalized against glyceraldehyde 3-phosphate dehydrogenase and transferrin). Poly A+ RNA from non-phosphaturic tumours and control tissues from OHO-patients (skin and material adjacent to tumours), CL8 human-renal cell line, human primary osteoblast cells (purchased from Clonetics H-OST, see materials), and poly A+ RNA extracted from a presumed tumour-polyp from a patient with linear sebaceous naevus syndrome (TCM from polyp did not inhibit phosphate uptake in human renal cell line CL8), did not amplify using MEPE specific primers. Using Clontech purchased cDNA's derived from heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas (human panel I #K1420-1), as templates for MEPE primer PCR, low level expression was detected in brain, liver, lung and pancreas. Sequencing of the MEPE-primer amplified bands revealed complete homology to MEPE cDNA and southern screening of the amplified bands with MEPE cDNA confirmed the sequencing results. OHO template poly A+ RNA from all OHO-patients consistently amplified an expected band of 480 bp and a lower band of 190 bp. The upper band was confirmed by sequencing and southern autoradiography as completely homologous to MEPE sequence, and the lower band was confirmed as a MEPE-derivative by southern analysis. The lower band did not appear in the low level expression normal-tissues or non OHO-tumours. This indicates that alternative splicing may play a role in the tumour derived MEPE. All RT/PCR and PCR experiments were normalized against G3PDH and transferrin.

In summary high level expression of MEPE (as measured by mRNA levels), was found only in OHO-tumour samples, and evidence for very low level expression (possibly ectopic), was found in brain, liver, kidney and three out of eleven non-OHO tumours. Eight out of eleven tumours were negative for MEPE mRNA

expression (RT/PCR), and all results were standardized against GA3PDH and transferrin RT/PCR primers,

Example 11: Southern analysis (Genomic blots)

Genomic blots containing immobilized DNA derived from a family with autosomal rickets (Rowe, Hum. Genet. 91 (1993), 571-575), and digested with PstI, EcoRI, PvuII, and MspI respectively were screened with radiolabeled MEPE cDNA as described above. Southern analysis was carried out using genomic digests of DNA extracted from blood as described previously (Rowe, Hum. Genet. 93 (1994), 291-294). The PstI blot revealed the presence of an 11 kb band and also a 4 kb polymorphism in one of the sixteen family members screened. The EcoRI, PvuII, and MspI blots were all positive for single bands of 6 kb, 6.5 kb, and 4 kb respectively, and confirmed the human provenance of the gene. Due to the lack of genetic information it was not possible to deduce whether the gene segregated with the disease in this autosomal rickets family.

Example 12: Phosphate uptake in a human renal cell line CL8: TCM and MEPE supplementation

Phosphate and glucose uptake experiments were conducted on a human renal cell line (CL8) as described previously (Rowe, Bone 18 (1996), 159-169). In brief cells were cultured in defined medium (DM), to confluency or overnight incubation in 24 well flat bottom tissue culture plates (Falcon 3047). The DM was then replaced with fresh DM supplemented with purified fusion protein or concanavilin affinity purified TCM and left overnight at 37° C. Uptake of P^{32} and C^{14} methyl-glucose was then measured (Rowe, Bone 18 (1996), 159-169).

Addition of TCM (1/20 dilution), to human renal cell lines resulted in a significant reduction in Na+ dependent phosphate uptake as reported earlier (Rowe, Bone 18 (1996), 159-169). This inhibition was prevented by preincubation of TCM with pre-operation and not post operation antisera, also reported earlier (Rowe, Bone 18 (1996), 159-169). Addition of high and low affinity concanavilin-A purified fractions (HCA and LCA respectively), at concentrations of 40 ng/ml also resulted in inhibition of Na+ dependent phosphate uptake (NaPi). In both TCM and concanavilin-A fractions the inhibition was specific to phosphate uptake, and did